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Regulation and Action of SKP2 in Cell and Tumor Models: Mechanisms Underlying Aggressive Growth in Basel-Like Breast Cancer

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14. ABSTRACT The objective of this research is to further our understanding of the molecular mechanisms underlying the aggressive growth of estrogen receptor (ER)-negative, basal-like breast tumors. My goal is to determine if SKP2 is a viable new therapeutic target to specifically treat patients who have tumors that are independent of ER signaling. The most significant result was the establishment of a TMX2-28 cell line that has been stably transfected with a SKP2-shRNA construct. This cell line will be instrumental in elucidating the role that SKP2 plays in breast cancer progression.					
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Introduction:

The objective of this research is to further our understanding of the cellular and molecular mechanisms underlying the aggressive growth of ER-negative, basal-like tumors. The goal is to identify new therapeutic targets to specifically treat patients that have tumors that are independent of ER signaling as these tumors are more often ER-negative. Past work from our lab and others has suggested that S-phase kinase-associated protein 2 (SKP2) plays an important role in breast tumorigenesis and would make a good therapeutic target. By utilizing three models (human tissue, animal models, and tissue culture) in which to characterize the role of SKP2 in breast cancer, we can obtain a better understanding of the molecular mechanisms underlying the aggressive tumor growth of basal-like breast tumors. It is anticipated that results from these studies will show that SKP2 would make a good therapeutic target for the treatment of women with basal-like tumors that are often associated with poor clinical outcome and tend to be ER-negative.

Body:

Task 1: During the second year of this three year study, I have obtained the necessary IRB approval to begin to collect archived formalin-fixed paraffin-embedded reductive mammaplaste and breast carcinoma tissues from the Department of Pathology, Baystate Medical Center. These tissues will be used to create tissue microarrays to be stained for SKP2, p27, phosphor-p27, CDK2, cyclin D1, and cyclin E.

Task 2: I have successfully created a mixed population, as well as a number of single clone populations of TMX2-28 cells that has been stably transfected with the negative control SKP2-shRNA vector. Additionally, I have successfully created a mixed population of TMX2-28 cells that have been stably transfected with SKP2-shRNA vector. Single clone populations of the SKP2-shRNA transfected cell line are currently being established. Once established, alterations in cell cycle can be assayed using FACS analysis.

Task 3: To date, I have isolated protein from untransfected TMX2-28, SKP2 knocked down TMX2-28, MCF-7, and MDA-MB-231 cells. Alterations in protein expression levels are currently being determined.

Key Research Accomplishments:

Training Accomplishments:

- Continue collaborations with **Dr. Christopher Otis**, Director of Surgical Pathology at Baystate Medical Center; **Dr. Brian Pentecost**, New York Department of Health;; **Dr. Sallie Smith-Schneider**, Pioneer Valley Life Sciences Institute; and **Dr. Douglas Anderton**, Associate Dean for Research Affairs, Director of Social and Demographic Research Institute
- Current and active member of AACR, AAAS, and SACNAS
- Continue to talk and meet with my mentor Dr. Kathleen Arcaro on a daily basis
- Attend weekly cancer and chemoprevention journal club, apoptosis journal club, molecular and cellular biology seminar and colloquia, animal biotechnology and biomedical science seminar

Research accomplishments:

- Obtained IRB approval from Baystate Medical Center to collect archived formalin-fixed paraffin-embedded reductive mammaplaste and breast carcinoma tissues
- Transfected TMX2-28 cells with SKP2-shRNA and successfully established a stably transfected cell line
- Isolated protein from TMX2-28, SKP2 knockdown TMX2-28, MCF-7, and MDA-MB-231 cell lines

Reportable Outcomes:

As a result of my research thus far, I have established a SKP2-shRNA stably transfected TMX2-28 cell line. This work has also led to the presentation of this research at the American Association of Cancer Research Advances in Breast Cancer Research Conference.

Conclusion:

The second year of this study has led to the acquirement of IRB approval, the establishment of a cell line, and the continuation of my training through collaborations and interactions with a number of clinicians, pathologists, bench scientists and epidemiologists. In the final year of this study I expect to complete all immunohistochemical, cell line establishment, gene/protein expression (task 3), cell cycle analysis, and *in vivo* work.

References: None

Appendices: American Association of Cancer Research Advances in Breast Cancer Research Conference Poster



S-Phase Kinase-Associated Protein 2 (SKP2) in Estrogen Receptor-Negative and Triple-Negative Breast Cancer

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Introduction

•Breast cancer is a heterogeneous disease that varies in its biology and response to therapy

•Basal-like tumors are associated with;

- A positive basal cytokeratin (CK) expression pattern (CK 5, 14 and/or 17)
- Negative expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)

•The objective of this research is to further our understanding of the cellular and molecular mechanisms underlying the aggressive growth of triple-negative/basal-like tumors

•S-phase kinase associated-protein 2 (SKP2) plays an important role in cell cycle regulation by targeting the cell cycle inhibitor p27 for degradation and subsequently promoting the progression into the S-phase of the cell cycle

•The novel cell line TMX2-28 was chosen as a model for ER-negative, basal-like breast cancer because this particular cell line is highly aggressive, ER-negative, has a mixed basal/luminal cytokeratin (CK) expression pattern and overexpresses SKP2

SKP2 Targets p27 for Degradation

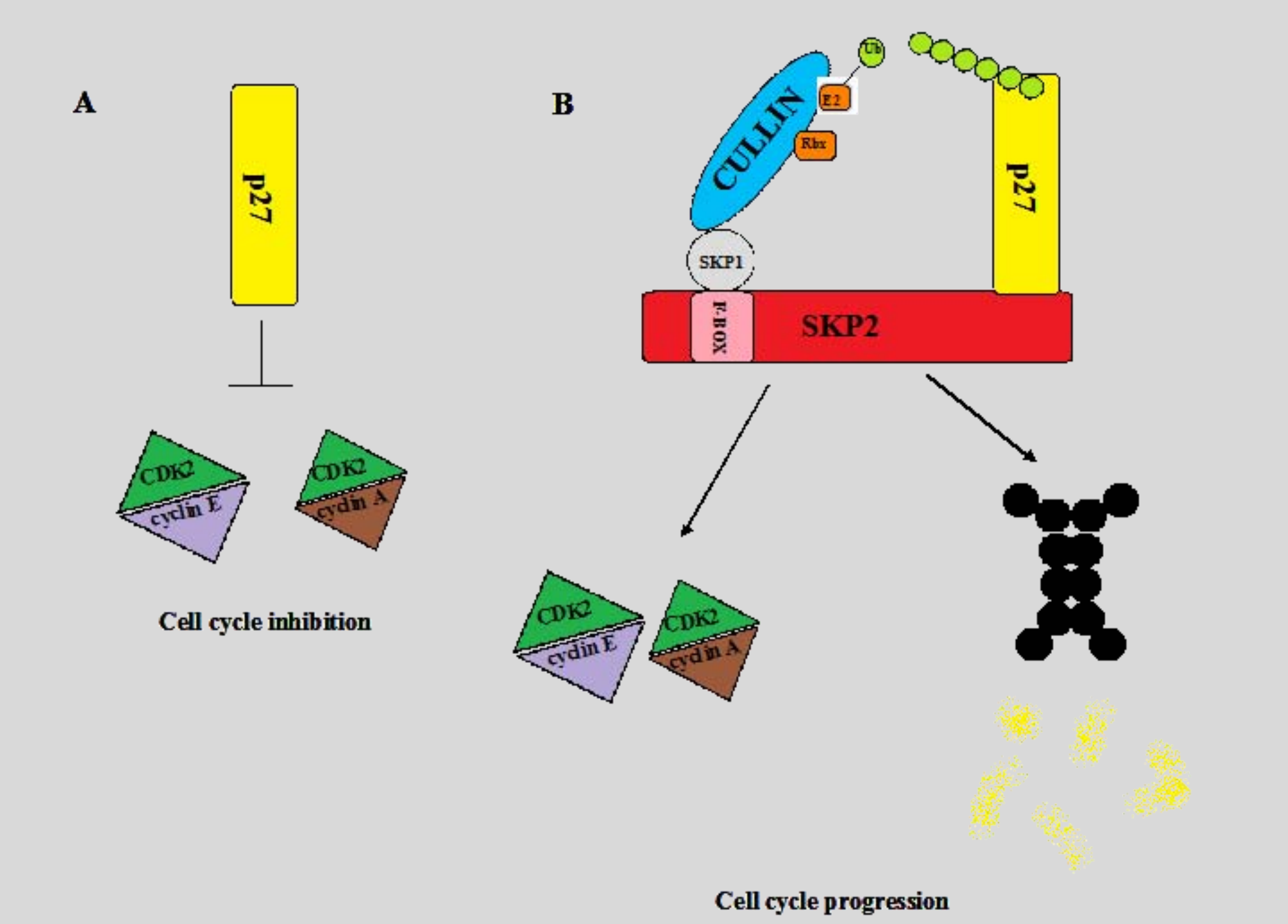


Figure 1: SKP2 promotes progression into the S-phase of the cell cycle by regulating p27. (A) p27 abrogates the actions of cyclin/CDK2 complexes thereby preventing the G₁-S transition and inhibiting the cell cycle. (B) In normal cells, SKP2 targets p27 for ubiquitin mediated degradation. By targeting p27 for degradation, SKP2 promotes progression into the S-phase of the cell cycle.

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TMX2-28 Cells Are Triple-Negative, Have a Mixed Basal/Luminal Cytokaratin mRNA Profile and Have an Altered Cell Cycle

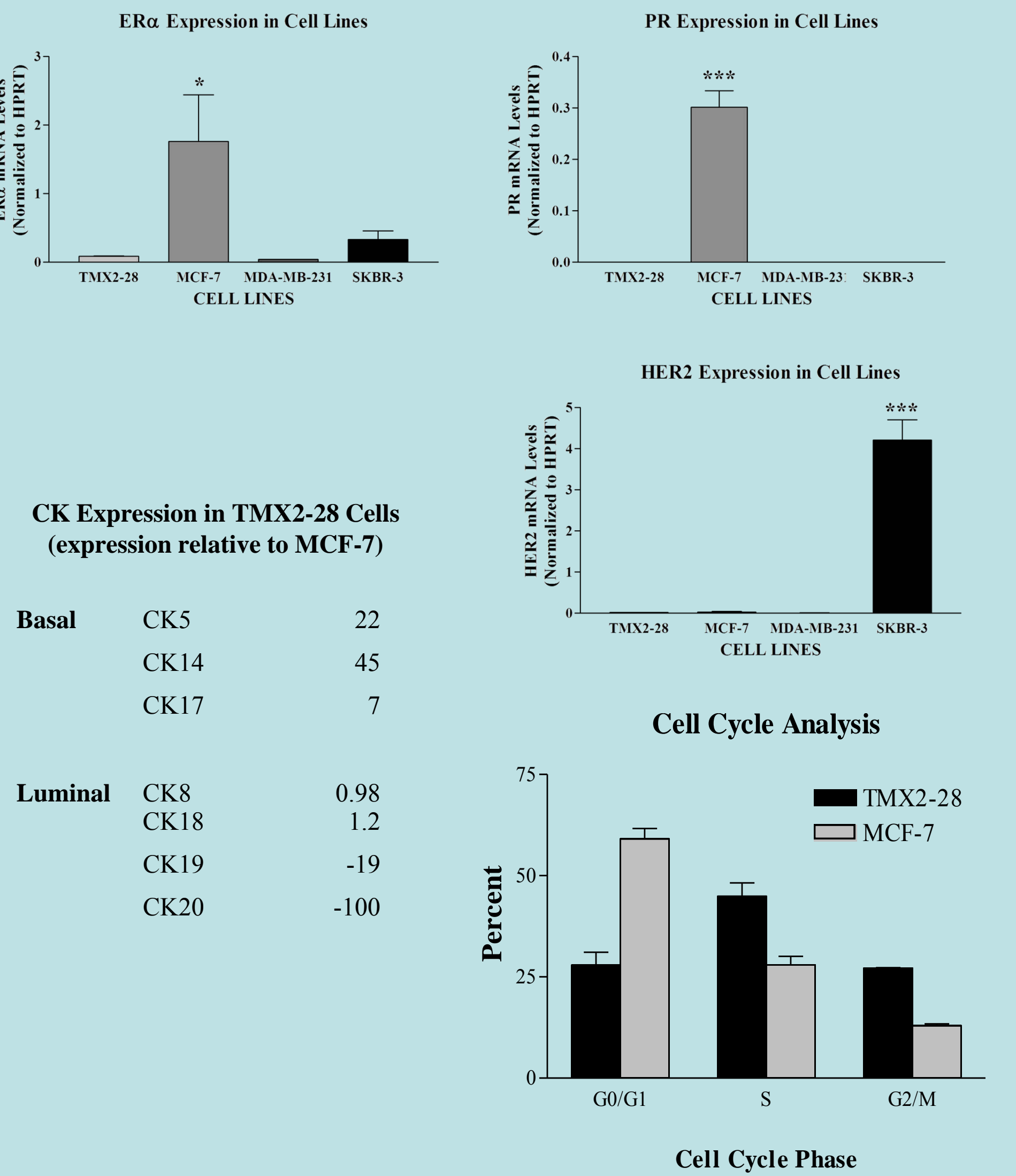


Figure 2: mRNA expression of ERα, PR, and HER2 was determined by real time qRT-PCR (One-way ANOVA p<0.0001). mRNA expression of seven CKs was examined by RT-PCR in TMX2-28 and compared to the expression profile of MCF-7 cells. Data are expressed as fold change in TMX2-28 compared to MCF-7 cells. Cell cycle analysis was determined by Flow Cytometry (FACS) analysis.

Cell Cycle Analysis

Percent

Cell Cycle Phase

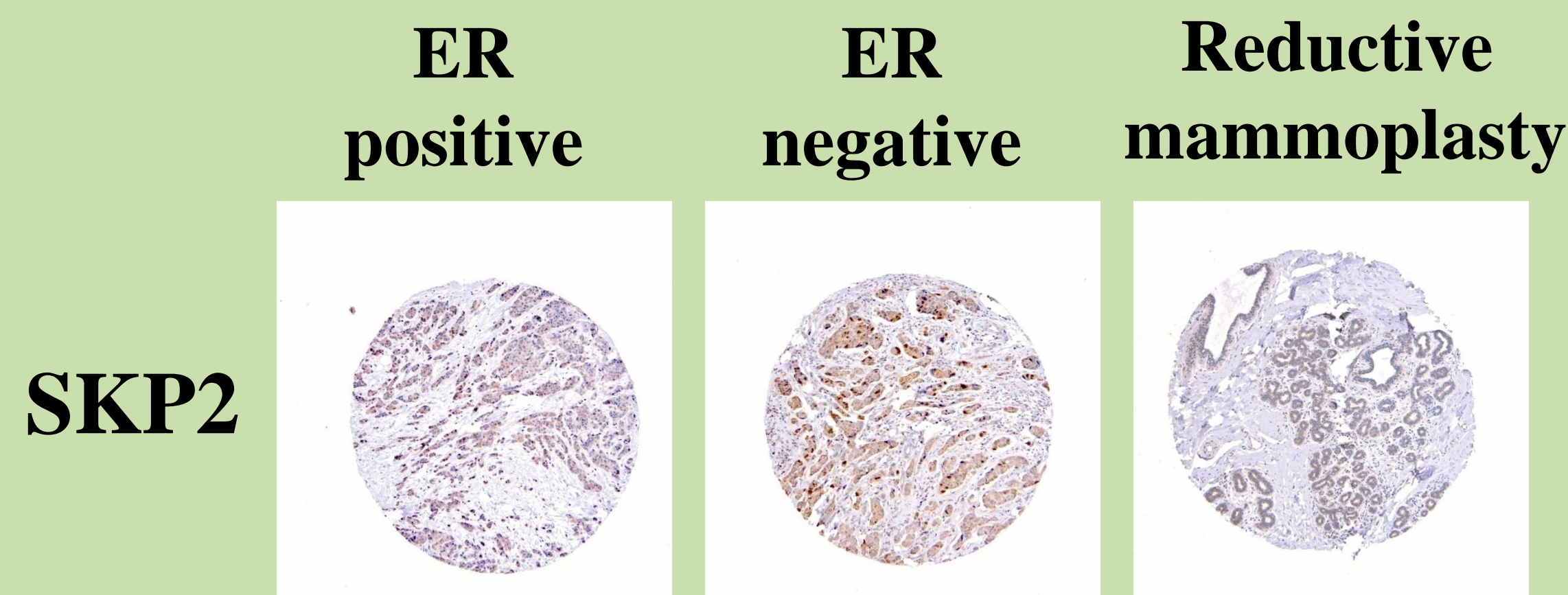
TMX2-28

MCF-7

Hypothesis

The highly proliferative and aggressive nature of ER-negative and triple-negative/basal-like breast cancer is the consequence of dysregulation of the cell cycle as a result of SKP2 overexpression.

SKP2 Protein is Highly Expressed in 46% of ER-Negative and 77% of Triple-Negative Breast Cancers



	SKP2 positive	SKP2 negative			SKP2 positive	SKP2 negative	
ER positive	23	73	96	Triple-negative	10	3	13
ER negative	17	20	37	Non-triple negative	24	75	99
	40	93			34	78	

Figure 4: SKP2 expression was examined in 37 ER-negative, 96 ER-positive, and 50 reductive mammoplasty tissue samples by immunohistochemistry. SKP2 was highly expressed in 46% (17 of 37) of ER-negative tumors, 24% (23 of 96) of ER-positive tumors, and 18% (9 of 50) reductive mammoplasty tissues. Importantly, SKP2 was highly expressed in 77% (10 of 13) of triple-negative breast cancers while only 24% (24 of 99) of non-triple negative breast cancers had high expression of SKP2. Representative SKP2 stained punches are shown.

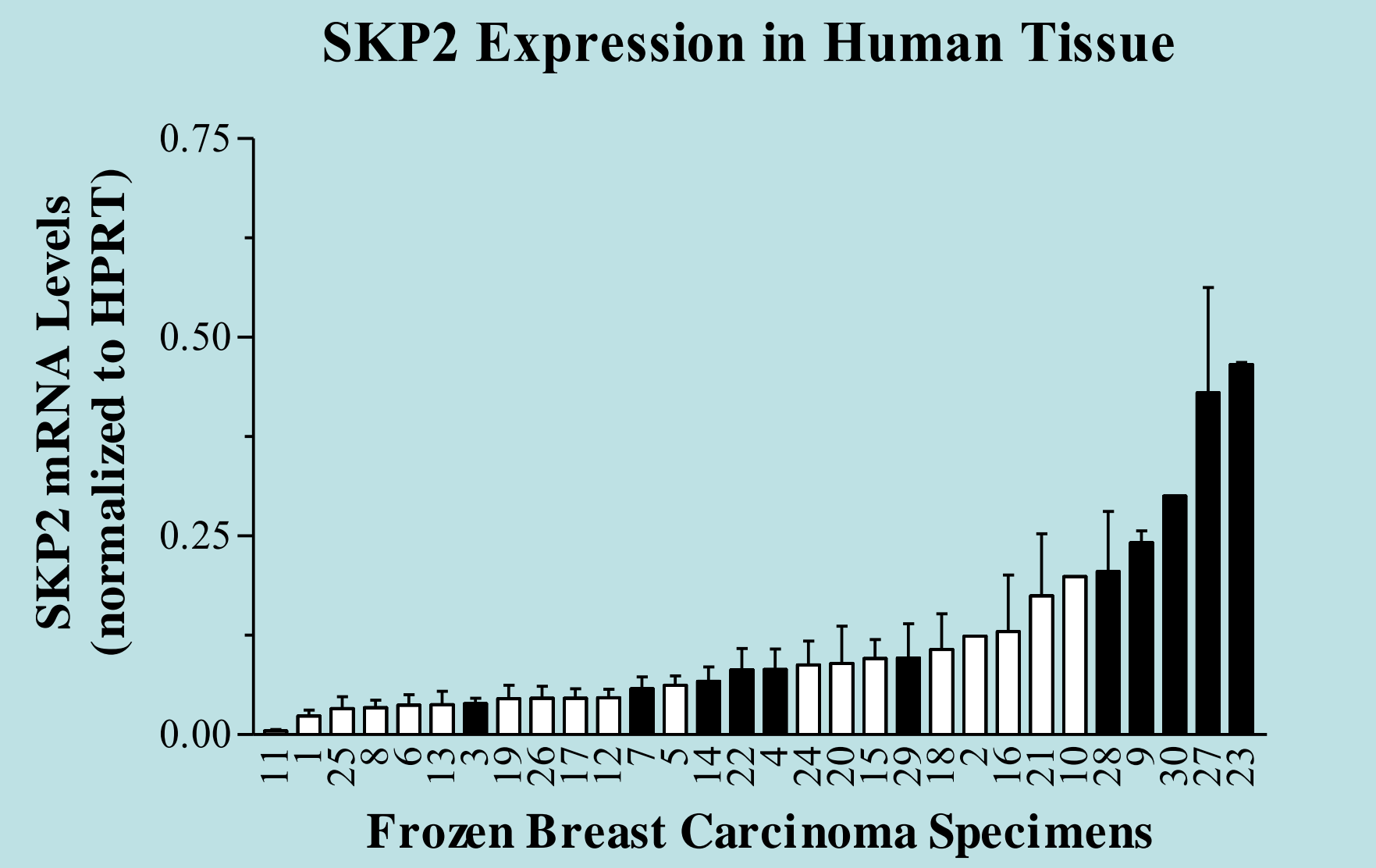
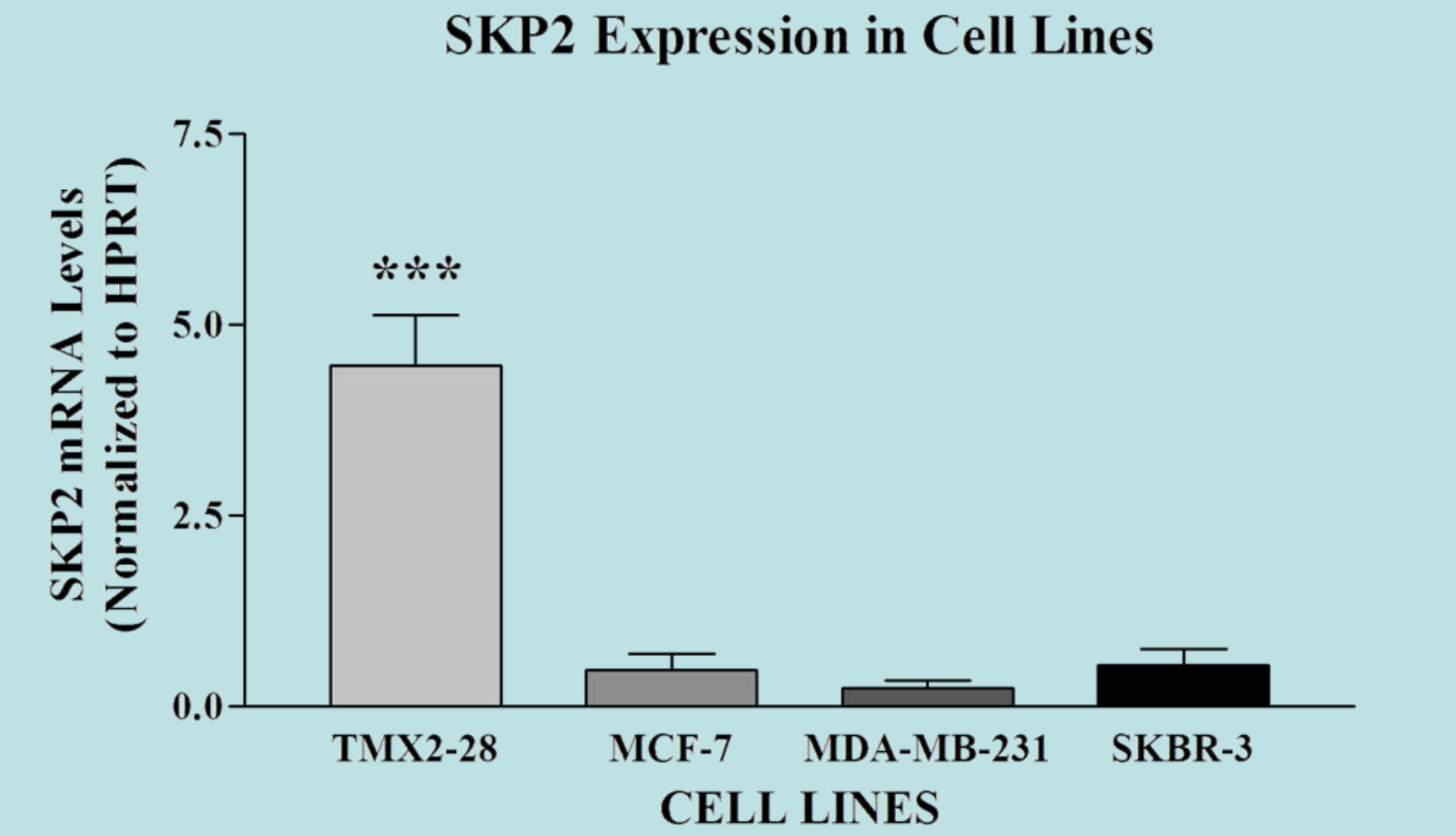


Figure 3: SKP2 mRNA expression in cell lines was determined using real time qRT-PCR (One-way ANOVA p<0.0001). Gene expression of SKP2 was determined in 30 frozen breast carcinoma samples using real time qRT-PCR. Tumors were sorted by SKP2 expression and correlated with CKs 5 and/or 17 positive and ER-negative tumors (Dark bars). ER-positive tumors were assigned the numbers 1-18 while ER-negative tumors were assigned 19-30.

TMX2-28 Cells Overexpress a Number of Cell Cycle Genes Associated with SKP2

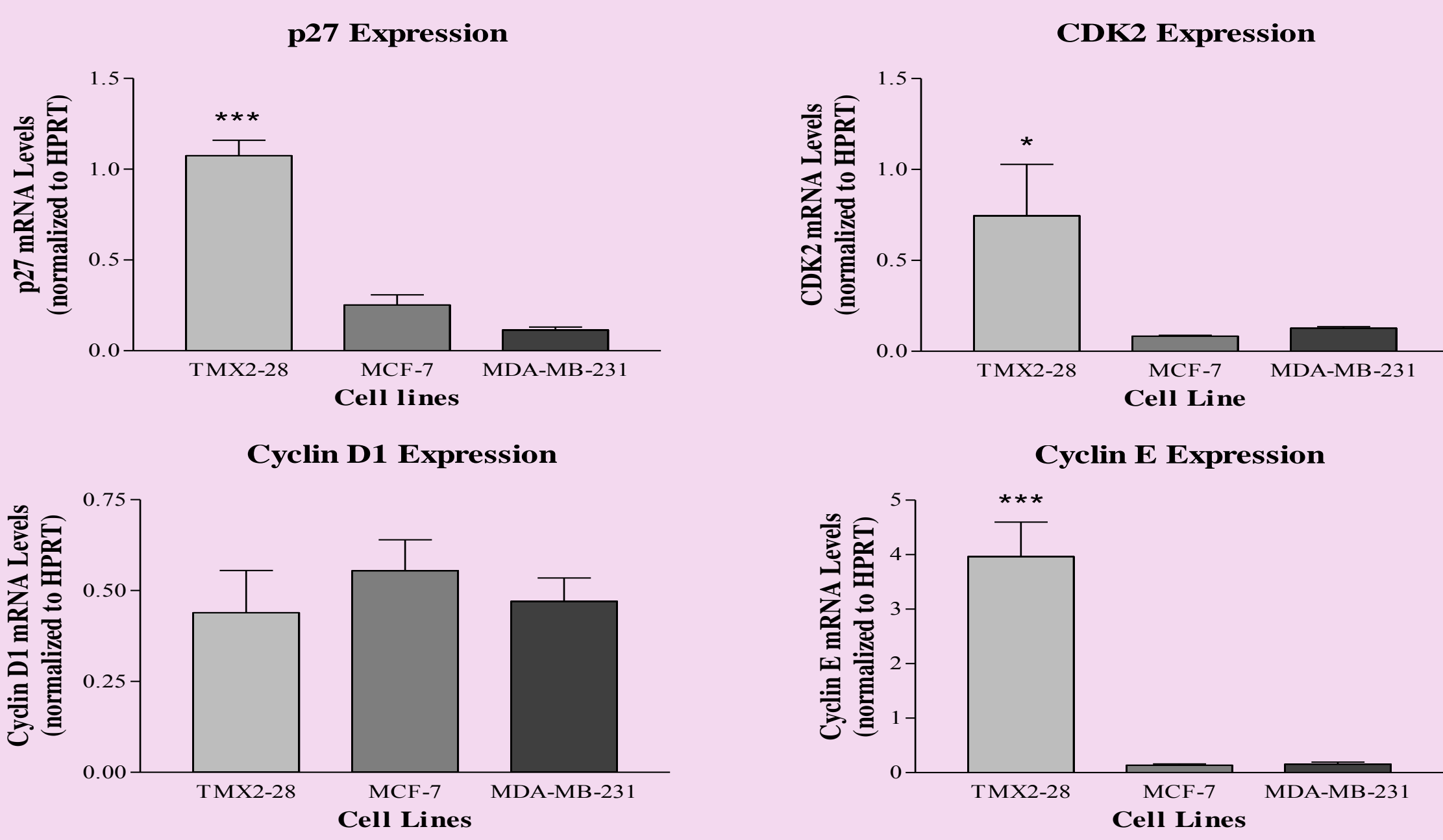


Figure 5: Gene expression was determined using real time qRT-PCR (One-way ANOVA, p27: p=0.0326; CDK2: p<0.0001; Cyclin E: p<0.0001).

Knockdown of SKP2 in TMX2-28 Cells does not Result in Significant Changes in the Gene Expression of the Cell Cycle Genes Associated with SKP2

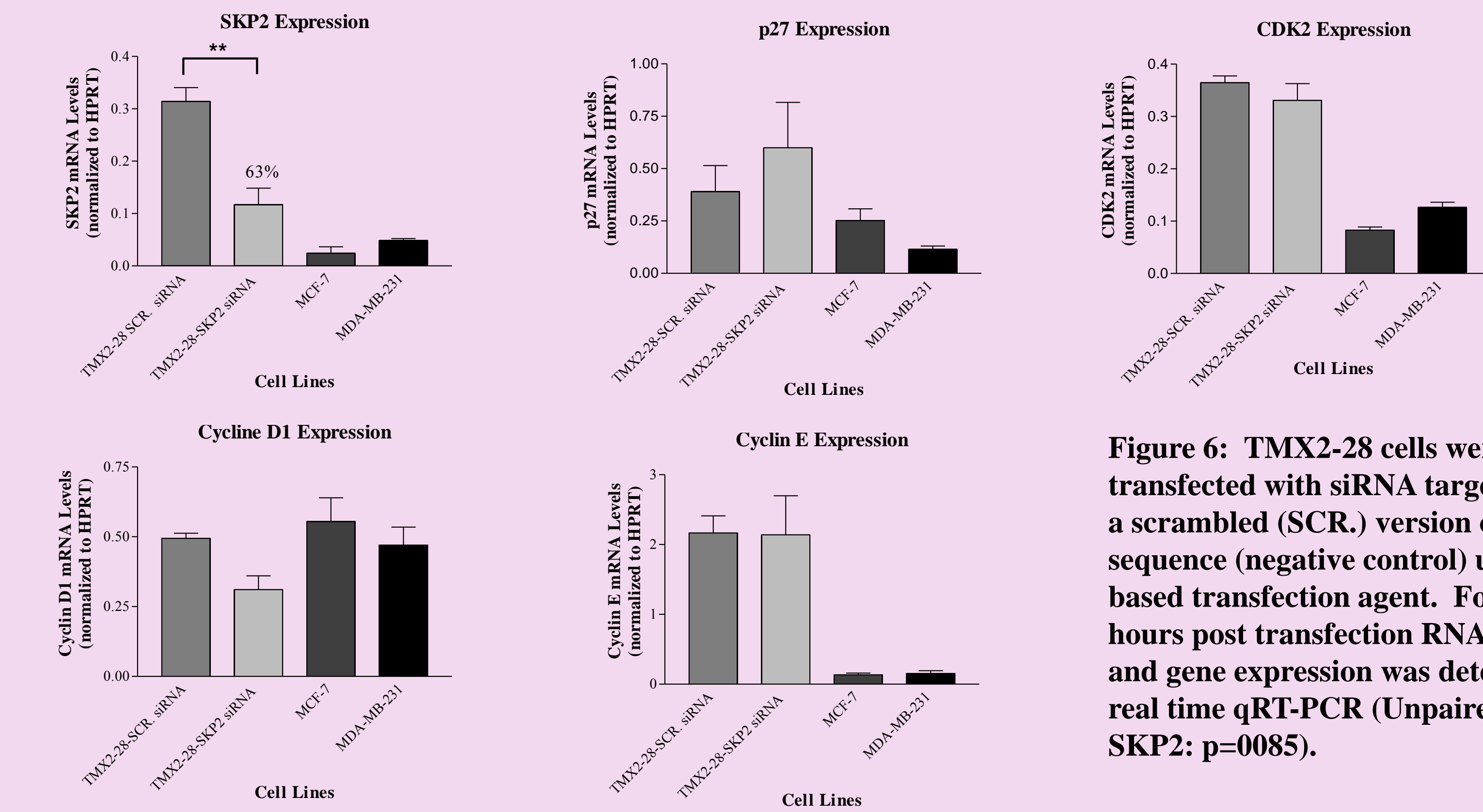


Figure 6: TMX2-28 cells were transiently transfected with siRNA targeting SKP2 or a scrambled (SCR.) version of the sequence (negative control) using a lipid based transfection agent. Forty-eight hours post transfection RNA was isolated and gene expression was determined using real time qRT-PCR (Unpaired T Test), SKP2: p=0085).

Summary

- TMX2-28 is a triple-negative breast cancer cell line that constitutively expresses a basal-like cytokeratin pattern and overexpresses SKP2
- TMX2-28 cells also overexpress a number of genes associated with SKP2 including p27, CDK2, and Cyclin E
- Knockdown of SKP2 expression by 63% does not significantly alter gene expression of its associated genes
- SKP2 was highly expressed in 46% (17 of 37) of ER-negative tumors, 24% (23 of 96) of ER-positive tumors, and 18% (9 of 50) reductive mammoplasty tissues
- SKP2 was also highly expressed in 77% (10 of 13) of triple-negative breast cancers while only 24% (24 of 99) of non-triple negative breast cancers had high expression of SKP2

Future Directions

- 300 additional ER-negative and triple-negative tumor cases to examine immunohistochemically
- Protein expression profiling of TMX2-28 cells pre- and post SKP2 knockdown
- Cell cycle analysis of TMX2-28 cells post SKP2 knockdown
- *in vivo* studies involving TMX2-28 cells where SKP2 has been stably knocked down